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The pH Dependence of the Association of β-Lactoglobulin

Robert Townend and Serge N. Timasheff

The pH Dependence of the Association of β-Lactoglobulin

The heterogeneity of crystalline preparations of β-lactoglobulin has been observed by many workers (1–4).[1] Recently, Ogston and Tilley (5) have reported the correlation of electrophoretic experiments, carried out as a function of temperature at pH 4.66, with some ultracentrifugal measurements. These authors concluded that β-lactoglobulin contains two species, one of which can undergo a reversible "dimerization" favored by low temperature, high protein concentration, and low pH.

An extensive ultracentrifugal study of β-lactoglobulin has been carried out in this laboratory as a function of pH, temperature, and protein concentration. The protein was obtained from Dr. W. Gordon who had prepared it from pooled milk whey by the method of precipitation with ammonium sulfate followed by repeated recrystallization from distilled water (6). The ultracentrifugal analyses were carried out in a Spinco Model E Analytical Ultracentrifuge at 59,780 r.p.m. The diagrams were analyzed from enlarged projected tracings in a manner similar to Ogston and Tilley (5). The apparent compositions reported are uncorrected for the Johnston and Ogston anomaly (7), since the magnitude of that correction was found to be within the precision of the area analyses in this system (ca. ±7%). It was found that, in agreement with Ogston and Tilley, the presence of an ultracentrifugally heavy component is contingent upon low temperatures and high protein concentrations. Measurements carried out at 2–3°C. in acetate buffer of 0.1 ionic strength on β-lactoglobulin solutions, 2.5–3.2 g./100 ml. in concentration (shown in Fig. 1), revealed little heavy component at pH 5.0, but a rapid increase in association down to pH 4.5. At this point the maximum extent of aggregation occurs with about 65% of the protein being in the rapidly sedimenting form. At lower pH values, however, the amount of heavy component falls off rapidly until at pH 3.5 the ultracentrifuge patterns reveal only slowly sedimenting material (Fig. 2).

From these data, it is clear that a reversible association is occurring. The $S_{20,w}$ values of the two components are 2.7–2.9 and 4.3–5.0 Svedberg units, respectively, suggesting that the heavy component is a β-lactoglobulin dimer, as proposed by Ogston and Tilley (5). It is interesting to note that the association occurs in a pH region in which β-lactoglobulin carries a net positive charge, while in its isoelectric region (pH 5.1–5.3) this protein is present completely in the form of a monomer.

Experiments carried out on a sample of β-lactoglobulin with an abnormally high percentage of electrophoretically rapid component[2] resulted in ultracentrifugal patterns with a larger proportion of heavy material. This would suggest that the heterogeneities observed by the two techniques are closely related. However, in view of the fact that in electrophoresis the heterogeneity is pronounced also at pH's above 5.1 and that prolonged (20–24 hr.) analyses reveal the presence of three components in the isoelectric zone,[1] it is not yet possible to identify unequivocally the components observed by the two techniques.

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[1] S. N. Timasheff, unpublished data.

[2] Seventy-five per cent; the normal preparation from pooled milk contains 60% of the rapidly migrating component (3).

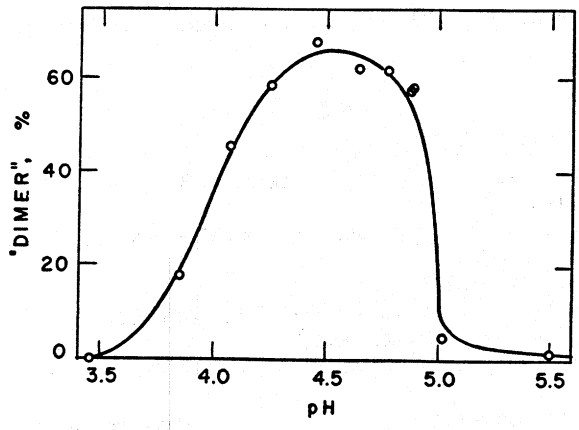
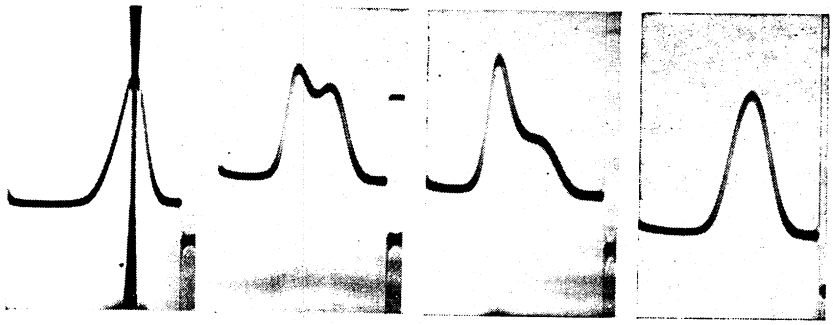


FIG. 1. Dependence of amount of β-lactoglobulin "dimer" on pH. Protein concentration 2.5–3.0 g./100 ml. Temperature: 2–3°C.; Acetate buffer: $\Gamma/2 = 0.1$.



pH 3.85 4.07 4.77 5.02

FIG. 2. Sedimentation diagrams of β-lactoglobulin at various pH's. Protein concentration: 3.0 g./100 ml., Temperature: 2.7°C.; 10,500 sec. after reaching full speed (sedimentation proceeds from right to left).

The pH dependence of the observed "dimerization" of β-lactoglobulin presents an unusual pattern suggestive of interaction between "specific sites" on the molecules of the protein. In order to elucidate the mechanism of this association, further ultracentrifugal and light-scattering studies as a function of the variables mentioned above, as well as of ionic strength and dielectric constant of the medium, are now in progress in this laboratory.

We would like to thank Dr. W. G. Gordon for supplying us with the β-lactoglobulin samples.

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Eastern Regional Research Laboratory,
Eastern Utilization Research Branch,
Agricultural Research Service,
U. S. Department of Agriculture,
Philadelphia, Pennsylvania
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Robert Townend
Serge N. Timasheff